Brief Genetics Report

Severe Persistent Hyperinsulinemic Hypoglycemia due to a De Novo Glucokinase Mutation

Antonio L. Cuesta-Muñoz,[1] Hanna Huopio,[2] Timo Otonkoski,[3] Juan M. Gomez-Zumaquero,[1] Kirsti Näntö-Salonen,[4] Jacques Rahier,[5] Soledad López-Enriquez,[1] Maria A. García-Gimeno,[6] Pascual Sanz,[6] Federico C. Soriguer,[1] and Markku Laakso[7]

Glucokinase (GK) is a glycolytic key enzyme that functions as a glucose sensor in the pancreatic β-cell, where it governs glucose-stimulated insulin secretion (GSIS). Heterozygous inactivating mutations in the glucokinase gene (*GCK*) cause a mild form of diabetes (maturity-onset diabetes of the young [MODY]2), and activating mutations have been associated with a mild form of familial hyperinsulinemic hypoglycemia. We describe the first case of severe persistent hyperinsulinemic hypoglycemia due to a "de novo" mutation in *GCK* (Y214C). A baby girl presented with hypoglycemic seizures since the first postnatal day as well as with inappropriate hyperinsulinemia. Severe hypoglycemia persisted even after treatment with diazoxide and subtotal pancreatectomy, leading to irreversible brain damage. Pancreatic histology revealed abnormally large and hyperfunctional islets. The mutation is located in the putative allosteric activator domain of the protein. Functional studies of purified recombinant glutathionyl S-transferase fusion protein of GK-Y214C showed a sixfold increase in its affinity for glucose, a lowered cooperativity, and increased k_{cat}. The relative activity index of GK-Y214C was 130, and the threshold for GSIS predicted by mathematical modeling was 0.8 mmol/l, compared with 5 mmol/l in the wild-type enzyme. In conclusion, we have identified a de novo *GCK* activating mutation that causes hyperinsulinemic hypoglycemia of exceptional severity. These findings demonstrate that the range of the clinical phenotype caused by *GCK* mutations varies from complete insulin deficiency to extreme hyperinsulinemia. *Diabetes* 53:2164–2168, 2004

From the [1]Hospital Carlos Haya Foundation and Department of Endocrinology, Diabetes, and Nutrition of Carlos Haya University Hospital, Málaga, Spain; the [2]Department of Paediatrics, University of Kuopio, Kuopio, Finland; the [3]Hospital for Children and Adolescents, Program of Developmental and Reproductive Biology, Biomedicum, University of Helsinki, Helsinki, Finland; the [4]Department of Paediatrics, University of Turku, Turku, Finland; the [5]Department d'Anatomie Pathologique, Cliniques Universitaires Sain Luc, Brussels, Belgium; the [6]Institute of Biomedicine of Valencia (CSIC), Valencia, Spain; and the [7]Department of Medicine, University of Kuopio, Kuopio, Finland.

Address correspondence and reprint requests to Antonio L. Cuesta-Muñoz, MD, PhD, The Department of Endocrinology, Diabetes and Nutrition of Carlos Haya Hospital, Hospital Civil, Plaza del Hospital Civil s/n, 29009 Málaga, Spain. E-mail: antoniol.cuesta.exts@juntadeandalucia.es.

Received for publication 3 December 2003 and accepted in revised form 4 May 2004.

A.L.C.-M. and H.H. contributed equally to this article.

GK, glucokinase protein; GSIS, glucose-stimulated insulin secretion; K_{ATP}, ATP-sensitive K^+; PHHI, persistent hyperinsulinemic hypoglycemia of infancy.

Failure of the pancreatic β-cell to suppress insulin secretion during hypoglycemia is the pathophysiological basis of persistent hyperinsulinemic hypoglycemia of infancy (PHHI). Mutations in the β-cell sulfonylurea receptor (*SUR1*) (1), the inward-rectifying potassium channel (*Kir6.2*) (2), along with mutations in glucokinase (*GCK*) (3,4), glutamate dehydrogenase (5), and short-chain 3-hydroxyacil-CoA dehydrogenase enzyme (6) genes are responsible for 50% of the cases of PHHI. Due to a high risk of permanent brain damage in these patients (7), an early diagnosis and efficient treatment are crucial. The clinically most severe cases are often due to mutations in the ATP-sensitive K^+ (K_{ATP}) channel genes, mainly *SUR1* (8). These patients often do not respond to treatment with diazoxide and in many cases require 95% subtotal pancreatectomy to prevent recurrent hypoglycemia (9).

Glucokinase (GK) is a glycolytic enzyme that functions as a "glucose sensor" in the pancreatic β-cell and governs glucose-stimulated insulin secretion (GSIS) (10). Inactivating mutations in *GCK* lead to the development of diabetes (11,12) and activating mutations to hyperinsulinism (3,4). Until now all activating mutations in the *GCK* that are described as causative of PHHI present a mild clinical phenotype with an excellent response to pharmacological treatment (3,4,13). In this report, we describe the first case of very severe PHHI due to a "de novo" mutation in *GCK*, refractory to both surgical and medical treatment and with a totally different clinical phenotype compared with other previously published PHHI cases linked to *GCK*. Our findings are important because they demonstrate that a mutation in *GCK* can also cause severe hyperinsulinism and that biochemical and structural changes in the GK protein are also responsible for the heterogeneity of the clinical phenotype of PHHI patients with activating *GCK* mutations.

We found a novel missense mutation in exon 6 of the *GCK* gene that resulted in the substitution of cysteine for tyrosine (Y214C). The proband was heterozygous for this mutation, and this variant was not found in any other family members or in 100 healthy control chromosomes. Since the paternity tests confirmed that the parents of the patient were her "biological parents," the mutation was considered as de novo. The structural analysis indicated

TABLE 1
Kinetic characteristics of the Y214C mutation of the *GCK* gene

	Wild type	Y214C
Glucose $S_{0.5}$ (mmol/l)	7.97 ± 0.64	1.24 ± 0.12
n_H	1.69 ± 0.12	1.32 ± 0.09
ATP_{Km} (mmol/l)	0.29 ± 0.04	0.60 ± 0.14
Turnover rate (s^{-1})	77.31 ± 7.61	164 ± 41.17
Catalytic efficiency ($k_{cat}/S_{0.5}$) ($s^{-1} \cdot mmol^{-1} \cdot l^{-1}$)	10.35 ± 1.65	132 ± 23.67
Relative activity index (I_{GKB})	1	130 ± 16.61

Data are means ± SE from three separate enzyme preparations. Results of functional studies of the enzyme carried out spectrophotometrically in the presence of 2 mmol of dithiothreitol per liter of reaction mixture. Glucose $S_{0.5}$ indicates the glucose concentration required for GK activity to be one-half maximal in the presence of a constant in excess concentration of ATP. The Hill coefficient (n_H) for cooperativity characterizes the sigmoidal glucose dependency of GK. ATP_{Km} indicates the ATP concentration required for GK activity to be one-half maximal when glucose is in excess. The turnover rate was obtained by increasing the glucose concentration in a stepwise manner in the presence of a constant concentration of ATP. The relative activity index is an expression of in situ phosphorylation capacity based on expression at 5 mmol/l of blood glucose and calculated according to the formula previously reported (19).

that Y214C is not located close to the glucose or ATP binding sites, but it is located in a loop in the suggested allosteric activator domain of GK (3,4,13), and the results of the kinetic analysis of the purified enzymes suggested that GK-Y214C was the cause of severe hyperinsulinism in our patient. GK-Y214C showed a sixfold higher affinity for glucose ($S_{0.5}$) and a twofold higher turnover rate (k_{cat}) when compared with wild-type GK (Table 1), and the cooperativity was almost completely lost. GK-Y214C also showed the highest activity index (130) of all naturally occurring activating *GCK* mutations described (3,4,13). This catalytic activation of GK-Y214C leads to an enzyme with a very low threshold for GSIS (~0.8 mmol/l, compared with 5 mmol/l for wild-type GK). Moreover, our kinetic results confirmed those observed (14) in the artificially made mutation, Y214A.

Recently, an allosteric activator of GK, RO-28-167, was described (15). Interestingly, the effects of GK-Y214C on k_{cat} and $S_{0.5}$ were similar to the effects of the GK activator RO-28-167 (Table 1), reinforcing the idea of the existence of an allosteric activator site in GK. Indeed, the crystal structure of GK recently developed (16) demonstrates that residue Y214 is located at the allosteric activator site and is included in the binding site of the synthetic activator used to crystallize the active form of GK. Interestingly, the decrease in the threshold for GSIS induced by the activator is less than that induced by Y214C (3 vs. 0.8 mmol/l, respectively). This is very important because to avoid frequent hypoglycemic episodes in the patient, the decrease in the GSIS threshold induced by the GK activator needs to be moderate. GK also seems to act as a glucose sensor in glucagon-producing pancreatic α-cells, in hepatocytes, in some hypothalamic neurons, and in some endocrine enterocytes, forming a neuroendocrine-signaling network that must be intact to maintain glucose homeostasis (17). Therefore, it is reasonable to assume that this GK signaling network was also affected in our patient. Both structural and functional changes in the GK-Y214C protein could explain the severity of hyperinsulinism and the differences in the clinical phenotype of our patient compared with other patients with activating mutations (3,4,13).

In contrast to the previously reported patients with activating *GCK* mutations, our patient was relatively large for gestational age. Macrosomia was presumably explained by the severe fetal hyperinsulinism consecutive to the Y214C mutation. If we consider that the mother of the boy with the GK-T65I mutation and the father of the boy with the GK-W99R mutation presented with normal birth weight, despite both having a de novo activating *GCK* mutation, it is interesting that previously reported (13) patients with PHHI due to activating mutations in *GCK* presented with normal birth weight.

The intravenous glucagon and adrenalin stimulation tests failed to increase blood glucose from low fasting levels, and leucine stimulation did not increase hypogly-

TABLE 2
Clinical studies of the patient with the Y214C activating mutation in the *GCK* gene: stimulation tests

	Adrenalin	Leucine	Glucagon (test 1)	Glucagon (test 2)
Blood glucose (mg/dl)				
0 min	25.2	23.4	25.2	27
15 min	–	19.8	–	–
30 min	34.2	21.6	46.8	45
60 min	37.8	21.6	9	27
120 min	32.4	–	27	18
180 min	28.8	–	21.6	21.6
240 min	–	–	23.4	21.6

Glucagon tests were performed at 2 months of age.

TABLE 3
Clinical studies of the patient with the Y214C activating mutation in the *GCK* gene: intravenous glucose tolerance test

	Blood glucose (mg/dl)	Serum insulin (pmol/l)	Growth hormone (ng/ml)
0 min	36	17.4	28
6 min	66.6	696	6.4
20 min	28.8	165.3	4.5
30 min	16.2	38.28	4.4
60 min	23.4	17.4	6.7
75 min	19.8	24.36	28.5

The intravenous glucose tolerance test was carried out at 5 months of age with a glucose dose of 0.5 g/kg body wt.

TABLE 4
Clinical studies of the patient with the Y214C activating mutation in the *GCK* gene: oral glucose tolerance test

	Blood glucose (mg/dl)		
	1.5 months of age	2.5 months of age (test 1)	2.5 months of age (test 2)
0 min	27	48.6	18
30 min	28.8	36	43.2
60 min	18	43.2	14.4
120 min	18	18	18
180 min	18	18	18
240 min	18	18	18

The oral glucose tolerance tests were carried out with a glucose dose of 1.75 g/kg body wt.

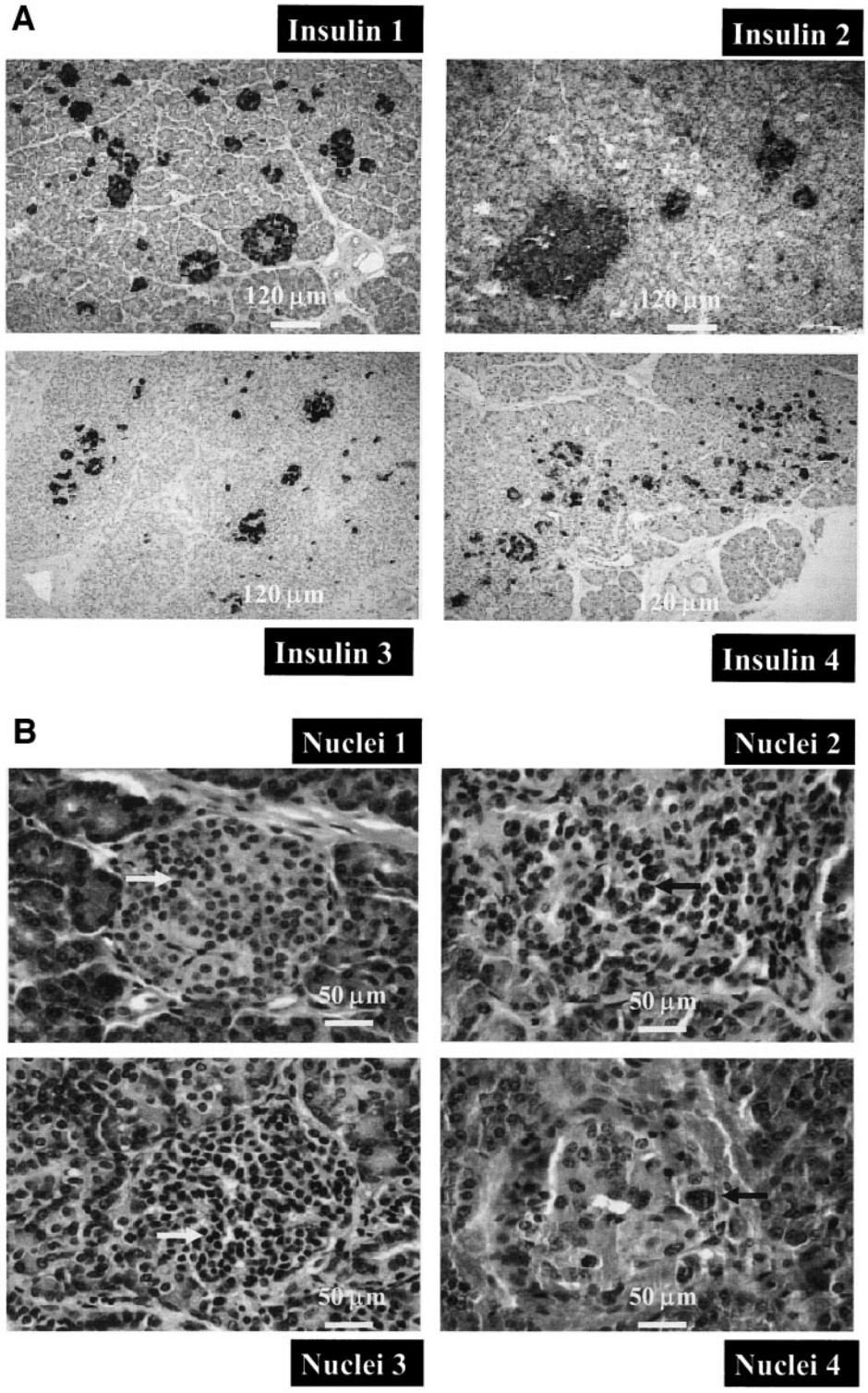

FIG. 1. ***A*****: Insulin immunostaining of pancreatic sections of a normal age-matched control subject (Insulin 1), of our patient with the GK-Y214C mutation (Insulin 2), of a patient with focal hyperinsulinism ("resting islet" outside a focal lesion) (Insulin 3), and of a patient with diffuse hyperinsulinism due to a homozygous SUR1 mutation (Insulin 4). The magnification (objective 10×) is identical in all cases.** ***B*****: Islet histology (Van Gieson staining) for pancreatic sections. The magnification (objective 40×) is identical in all cases. Black arrows indicate large nuclei. The sizes of 100 islet profiles were measured on insulin-stained sections with the semiautomatic image analyzer (KS400) coupled with an Axiocam camera (Zeiss, Thornwood, NY).**

cemia, showing the absence of hypersensitivity to this amino acid (Table 2). The low threshold for GSIS induced by the Y214C mutation may explain the results of the intravenous glucose tolerance test in our patient, where insulin secretion was high even at blood glucose concentrations as low as 16.2 mg/dl (Table 3). In contrast to other cases of PHHI related to activating mutations in *GCK*, no glycemic response whatsoever was present in the oral glucose tolerance test results of our patient. In fact, plasma glucose levels were always low, decreasing rapidly until glycemia reached the pathologic value of 18 mg/dl (Table 4). Because GK also serves as a metabolic glucose sensor in pancreatic α-cells (18), this implies that our patient had to have a very low threshold for glucose-stimulated glucagon secretion as well, leading to a severe delay in glucagon secretion. The lack of glucagon-stimulated glucose increase and the irregular response of growth hormone in the intravenous glucose tolerance test

in our patient show, in contrast to other PHHI patients having activating mutations in *GCK*, an altered counterregulatory response that could further aggravate severe hypoglycemia.

Our patient, like those with *GCK* activating mutations previously reported, exhibited a normal lipid profile. However, only our patient had hepatomegaly and cardiomegaly. The accumulation of excessive amounts of glycogen in liver could explain the hepatomegaly, and increased glycogen synthesis in muscle induced by hyperinsulinism could explain the cardiomegaly.

Histological changes in the pancreas also differentiate our patient from other previously reported patients with activating *GCK* mutations. Although histology was normal in the previously reported cases (3,4,13), the average size of islet profiles was ~2.5-fold in our patient compared with that of control subjects, and 10- and 8-fold compared with those of islets from diffuse or focal PHHI patients, respectively (Fig. 1*A*). This increase in size is probably due to increased insulin secretion from the β-cells. Some β-cells had abnormally large nuclei, but these were clearly smaller and less frequent than in patients with a typical SUR1-related diffuse form (Fig. 1*B*). Islet nuclear crowding (the number of endocrine nuclei per cytoplasm area) was intermediate between diffuse PHHI and control or focal PHHI subjects. The absence of proinsulin cytoplasmic labeling indicates that proinsulin was normally cleaved at the end of the Golgi apparatus. Increased labeling in the Golgi area reflects a high level of insulin synthesis. However, this was accompanied by low-intensity insulin immunostaining, indicating decreased insulin storage associated with continuous high insulin secretion (Fig. 1*A* and *B*).

In PHHI, a subtotal pancreatectomy may be necessary to control hypoglycemia in patients who do not respond to diazoxide. Neither subtotal pancreatectomy nor the treatment with diazoxide resolved hypoglycemia of our patient. We can assume that the high turnover and the very low threshold for GSIS induced by GK-Y214C allows high insulin secretion, even with only 5% of the pancreas left, particularly because the infantile pancreas may undergo significant regeneration (19). The absence of response to diazoxide is a characteristic feature shared by some patients with severe familial hyperinsulinism due to mutations in the K_{ATP} channel that are often completely unresponsive to diazoxide. Theoretically, the hyperinsulinism of our patient should have responded to diazoxide. This unresponsiveness could be due to the low dose used, which was not sufficient to increase the threshold for GSIS enough to overcome the low threshold provided by Y214C.

In conclusion, we describe here a new type of very severe hyperinsulinism caused by a de novo mutation in *GCK* with unique clinical characteristics compared with previously described activating *GCK* mutations. Our findings are important for several reasons. First, they demonstrate the role of GK as the most important "glucostat" of the human body. Second, they indicate that the target of the GK activator discovered may be the allosteric heterotropic activator site, and the amino acid residue Y214 could be part of it. Third, they prove that the clinical phenotype of PHHI patients with activating mutations in *GCK* is not homogeneous but rather heterogeneous. Finally, they indicate that genetic and functional studies of GK should also be considered in severe cases of familial hyperinsulinemic hypoglycemia.

RESEARCH DESIGN AND METHODS

After a normal pregnancy, a baby girl of Finnish ancestry was born full term in 1972. She was relatively large for gestational age, with a weight and height at birth of 4,350 ± 1.8 g (±SD) and 52 ±1 cm, respectively. She had intrapartum asphyxia, with an Apgar score of 5/7. Since the first postnatal day, she presented symptoms of nonketotic hypoglycemia, with cyanosis, shivering, unconsciousness, and convulsions. Cardiomegaly, hepatomegaly, hypocalcemia, and hypomagnesemia were detected. Blood glucose levels were permanently hypoglycemic (0.1–2.6 mmol/l), independent of feeding. Despite a leucine-free diet, high-dose glucose infusion, and hydrocortisone and glucagon treatment, hypoglycemia persisted. She was treated with diazoxide ($2\ mg \cdot kg^{-1} \cdot day^{-1}$), with no response. At the age of 6 months, the baby underwent a subtotal pancreatectomy (95%), with no resolution of hypoglycemia. By the age of 10 months, signs of developmental retardation were evident and her electroencephalogram was severely abnormal. Until her death at the age of 29 years, she had severe mental retardation and epilepsy. She was on diazoxide therapy (2–5 mg/kg) for 22 years. During this period, glucose levels were always between 0.5 and 2.4 mmol/l. At the age of 22 years, diazoxide treatment was discontinued, without any effect on glycemia (1.6–2.9 mmol/l). There was no family history of diabetes or other disorders related to glucose metabolism. Her two healthy sisters had normal development.

Metabolic studies. The studies, performed according to the Declaration of Helsinki, were approved by the institutional ethics committee, and written informed consent was obtained from the parents of the proband. An intravenous glucagon test, adrenalin and leucine stimulation tests, an oral glucose tolerance test, and an intravenous glucose tolerance test were performed before subpancreatectomy.

Genetic studies. The promoter region, all 39 exons and flanking introns of the *SUR1* gene, the single exon of the *Kir6.2* gene, exons 11 and 12 of the glutamate dehydrogenase gene, and the promoter region and all 10 exons of the *GCK* gene were screened for mutations with the single-strand conformation polymorphism analysis in the proband. The variant of the *GCK* gene in exon 6 detected by single-strand conformation polymorphism analysis was identified by direct sequencing and verified by digestion with restriction endonuclease *Bbv*I. Paternity tests in the proband and her parents involved the determination of common polymorphisms in 10 different genes and 19 polymorphisms in the HLA-DQB1 region.

Histological analysis. Histological analysis was performed on the surgically removed specimens when the patient was 6 months old and not on the remnant pancreas after the patient died. Pancreatic specimens were fixed in formalin and embedded in paraffin. The 7-μm thick sections were stained with the Van Gieson technique or were incubated with a monoclonal anti-insulin antibody (dilution 1/1,000; Chemicon, Temecula, CA) or with a monoclonal proinsulin antibody (dilution 1/500; Novobiolab, Copenhagen, Denmark), both of which were detected and revealed by a secondary anti-mouse antibody and alkaline phosphatase anti-alkaline phosphatase complex (Dako, Carpinteria, CA).

Functional studies of the protein: site-directed mutagenesis, kinetic, and structural analysis. The mutation of the *GCK* gene and wild type were expressed in *Escherichia coli* to carry out the purification. Kinetic studies of the wild-type GK and GK-Y214C were performed spectrophotometrically, as previously described (20). We used nonlinear kinetics according to the Hill equation, to investigate the affinity of the enzyme for glucose, and the Hill coefficient, which characterizes the sigmoidal glucose dependency of GK. After making at least three different preparations of both wild type and GK-Y214C, the results obtained were compared. To measure glucose phosphorylation capacity of the enzyme, we used the relative activity index. Structural analysis was done using the structural model of human GK as previously described (21,16). We used a minimal mathematical model that has been previously described (10) to assess the impact of the *GCK* mutation on GSIS.

ACKNOWLEDGMENTS

This work was supported by Spanish Ministry of Science and Technology grant no. CYT-836, by a Novo Nordisk grant, by SAF2001/3623 (all to A.L.C.-M.), and by grant BMC2002-00208 (to P.S.). The FIS of the Instituto de Salud Carlos III, Madrid, Spain, grant no. 02/PI021473 was awarded to A.L.C.-M. and the RCMN (C03/08). Grants from the Foundation of Pediatric Research were awarded to

T.O., and the Academy of Finland and the European Union (grant no. QLG1-CT-1999-00674) provided grant support to M.L.

REFERENCES

1. Thomas PM, Cote GJ, Wohllk N, Haddad B, Mathew PM, Rabl W, Aguilar-Bryan L, Gagel RF, Bryan J: Mutations in the sulfonylurea receptor gene in familial hyperinsulinemic hypoglycemia of infancy. *Science* 268: 426–429, 1995
2. Thomas P, Ye Y, Lightner E: Mutation of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. *Hum Mol Genet* 5:1809–1812, 1996
3. Glaser B, Kesavan P, Heyman M, Davis E, Cuesta A, Buchs A, Stanley CA, Thornton PS, Permutt MA, Matschinsky FM, Herold HC: Familial hyperinsulinism caused by an activating glucokinase mutation. *N Engl J Med* 338:226–230, 1998
4. Christensen HB, Jacobsen BB, Odili S, Buettger C, Cuesta-Munoz A, Hansen T, Brusgaard K, Magnuson MA, Shiota C, Matschinsky FM, Barbetti F: The second activating mutation (A456V): implications for glucose homeostasis and diabetes therapy. *Diabetes* 51:1240–1246, 2002
5. Stanley CA, Lieu YK, Hsu BY, Ming JE, Glaser B, Poncz M: Hyperinsulinism and hyperamonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *N Engl J Med* 338:1352–1357, 1998
6. Clayton PT, Eaton S, Aynsley-Green A, Edginton M, Hussain K, Krywawych S, Datta V, Malingré HEM, Berger R, van den Berg IET: Hyperinsulinism in short-chain l-3-hydroxyacyl-CoA dehydrogenase deficiency reveals the importance of β-oxidation in insulin secretion. *J Clin Invest* 108:457–465, 2001
7. Duvanel CB, Fawer CL, Cotting J, Hohlfeld P, Matthieu JM: Long-term effects of neonatal hypoglycemia on brain growth and psychomotor development in small-for-gestational-age preterm infants. *J Pediatr* 134: 492–498, 1999
8. Glaser B: Hyperinsulinism of the newborn. *Semin Perinatol* 24:150–163, 2000
9. Otonkoski T, Ammala C, Huopio H, Cote GJ, Chapman J, Cosgrove K, Ashfield R, Huang E, Komulainen J, Ashcroft FM, Dunne MJ, Kere J, Thomas PM: A point mutation inactivating the sulphonylurea receptor causes the severe form of persistent hyperinsulinemic hypoglycemia of infancy in Finland. *Diabetes* 48:408–415, 1999
10. Matschinsky FM: Regulation of pancreatic β-cell glucokinase: from basics to therapeutics. *Diabetes* 51 (Suppl. 3):S394–S404, 2002
11. Froguel P, Zouali H, Vionnet N, Velho G, Vdaire M, Sun F, Lesage S, Stoffel M, Takeda J, Passa P, Permutt A, Beckmann JS, Bell GI, Cohen D: Familial hyperglycemia due to mutations in glucokinase. *N Engl J Med* 328:697–702, 1993
12. Njolstad PR, Sovic O, Cuesta-Munoz A, Bjorkhaug L, Massa O, Barbetti F, Undlien DE, Shiota C, Magnuson MA, Molven A, Matschinsky FM, Bell GI: Neonatal diabetes mellitus due to complete glucokinase deficiency. *N Engl J Med* 344:1588–1592, 2001
13. Gloyn AL, Noordam K, Willemsen MA, Ellard S, Lam WWK, Campbell IW, Midgley P, Shiota C, Buettger C, Magnuson MA, Matschinsky FM, Hattersley AT: Insights into the biochemical and genetic basis of glucokinase activation from naturally occurring hypoglycemia mutations. *Diabetes* 52:2433–2440, 2003
14. Moukil MA, Veiga-da-Cunha M, Van Schaftingen E: Study of the regulatory properties of glucokinase by site-directed mutagenesis: conversion of glucokinase to an enzyme with high affinity for glucose. *Diabetes* 49:195–201, 2000
15. Grimsby J, Sarabu R, Corbett WL, Haynes NE, Bizzarro FT, Coffey JW, Guertin KR, Hilliard DW, Kester RF, Mahaney PE, Marcus L, Qi L, Spence CL, Tengi J, Magnuson MA, Chu CA, Dvorozniak MT, Matschinsky FM, Grippo JF: Allosteric activators of glucokinase: potential role in diabetes therapy. *Science* 301:370–373, 2003
16. Kenji K, Morihiro M, Teruyuki N, Jun-ichi E, Yasufumi N: Structural basis for allosteric regulation of the monomeric allosteric enzyme human glucokinase. *Structure* 12:429–438, 2004
17. Bell GI, Cuesta-Munoz A, Matschinsky FM: Glucokinase. In *Encyclopedia of Molecular Medicine.* New York, John Wiley & Sons, 2002, p. 1437–1442
18. Heimberg H, De Vos A, Moens K, Quartier E, Bouwens L, Pipeleers D, Van Schaftingen E, Madsen O, Schuit F: The glucose sensor protein glucokinase is expressed in glucagon-producing cells. *Proc Natl Acad Sci U S A* 93:7036–7041, 1996
19. Schönau E, Deeg KH, Huemmer HP, Akcetin YZ, Böhles HJ: Pancreatic growth and function following surgical treatment of nesidioblastosis in infancy. *Eur J Pediatr* 150:550–553, 1991
20. Davis EA, Cuesta-Muñoz A, Raoul M, Buettger C, Sweet I, Moates M, Magnuson MA, Matschinsky FM: Mutants of glucokinase cause hypoglycaemia and hyperglycaemia syndromes and their analysis illuminates fundamental quantitative concepts of glucose homeostasis. *Diabetologia* 42:1175–1186, 1999
21. Mahalingam B, Cuesta-Munoz A, Davis EA, Matschinsky FM, Harrison RW, Weber IT: Structural model of human glucokinase in complex with glucose and ATP: implications for the mutants that cause hypo- and hyperglycemia. *Diabetes* 48:1698–1705, 1999